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Anxiolytic-like effects of leptin on fixed interval responding

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ABSTRACT

Leptin has been shown to affect energy homeostasis, learning and memory, and some models of anxiolytic action. However, leptin has produced inconsistent results in previous non-operant behavioural tests of anxiety. Here, we test the anxiolytic potential of leptin in an operant paradigm that has produced positive results across all classes of anxiolytic so far tested. Rats were tested in the Fixed Interval 60 Seconds (FI60) task following administration of 0/0.5/1.0 mg/kg (i.p.) leptin or an active anxiolytic control of 5 mg/kg (i.p.) chlordiazepoxide (CDP). By the end of the 14 days of testing in the FI60 task, 0.5 mg/kg leptin released suppressed responding in a manner similar to CDP, and 1.0 mg/kg leptin produced a relative depression in responding, a similar outcome pattern to previously tested 5HT-agonist anxiolytics. This suggests that leptin behaves similarly to established serotonergic anxiolytics such as buspirone and fluoxetine; with the delay in development of effect during testing, and the inverted-U dose–response curve explaining the inconsistent behaviour of leptin in behavioural tests of anxiety, as this type of pattern is common to serotonergic anxiolytics.

1. Introduction

Leptin is released primarily from adipocytes in response to the ingestion of dietary fat (Havel, 2000). It has long been thought to be a feedback signal involved in satiety, but has since been shown to have diverse roles unrelated to energy regulation (Margetic et al., 2002). One of these novel roles for leptin is to act centrally on some aspects of cognition and anxiety — functions typically thought to be controlled by the hippocampus (Asakawa et al., 2003; Farr et al., 2006; Gisou et al., 2009; Paulus et al., 2005).

There is substantial behavioural evidence linking endogenous leptin to anxiety. Genetically modified ob/ob mice, which fail to produce leptin, display more anxious behaviour (Finger et al., 2010) and the administration of exogenous leptin reverses this difference (Asakawa et al., 2003). They also, like animals treated with anxiolytic drugs (McNaughton and Morris, 1987, 1992), show impaired spatial memory (Farr et al., 2006), implicating structures involved in the regulation of anxiety, such as the hippocampus, in the action of leptin. Conversely, maternal hyperleptinaemia, via injection of leptin while pups are in utero, produces adult rats that display improved spatial memory and reduced anxious behaviour compared to animals whose dams were injected with vehicle (Fraga-Marques et al., 2010).

There is also evidence for interaction of exogenous leptin with the control of anxiety. The Hypothalamic–Pituitary–Adrenocortical (HPA) axis is a central structure in anxiety (Faravelli et al., 2012; Landgraf et al., 1999). Leptin has been shown to diminish hyperactivity of the HPA axis (Holmes, 2015; Perry et al., 2014) and activation of neurons expressing the leptin receptor (LepRb) in the lateral hypothalamic area results in the inhibition of HPA axis activity (Bonnavion et al., 2015). Leptin is so central to this system that it has been included in the HPA axis as the Hypothalamic–Pituitary–Adrenal–Leptin axis (Aschbacher et al., 2014). It is also possible that leptinergic modulation of this axis is through 5-HT (Guo and Lu, 2014; Haleem et al., 2015; Kurhe et al., 2015). Leptin can also behave like established anxiolytics in some behavioural tests of anxiety (Liu et al., 2010; Wang et al., 2015).

The picture in relation to exogenous anxiolytic action is not homogeneous, however. Several experiments have produced non-positive results. Suomalainen and Mannisto (1998) failed to obtain changes in anxiety-related behaviour in the elevated plus maze or open field. Buyse et al. (2001) found that leptin significantly reduced the number of open-arm entries in the elevated plus maze, as did Thorsell et al. (2002). In contrast to these anxiogenic-like findings in the elevated plus maze, Liu et al. (2010) showed that leptin administration produced anxiolytic-like effects in the elevated plus maze, but not in the open field test. Both the elevated plus maze and open field test are standard behavioural tests of anxiety. Hogg (1996) points out that the variability in response to the elevated plus maze, particularly in response to serotonergic agonists, is highly contingent on a variety of factors such as the aversiveness of the testing environment and the prior handling experience of the animals in the tests. There was also considerable variation in the dose of leptin used in these tests; Suomalainen and

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Mannisto (1998) used 10 and 20 mg/kg, Buyse et al. (2001) 0.4–1 mg/kg, and Liu et al. (2010) 0.25 and 1 mg/kg. In contrast to the i.p. administration in the other studies, Thorsell et al. (2002) delivered 0.1 mg/kg intraventricularly.

While thought to bind primarily to the LEPR-encoded leptin receptor family there is evidence that leptin modulates, and is modulated by, the activity of 5-HT (Garcia-Alcocer et al., 2010; Morrison, 2004), perhaps in an indirect manner. 5-HT is a key neurotransmitter system that is involved in the anxiolytic function of serotonergic anxiolytics such as buspirone (Riblet et al., 1982) and fluoxetine (Fuller et al., 1991). Administration of leptin increases brain serotonin (5-HT) metabolism in mice (Calapai et al., 1999). There is also evidence that administration of the 5-HT precursor 5-hydroxytryptophan significantly increases serum leptin levels (Yamada et al., 1999); and serotonergic neurons have been shown to be targets for leptin in the monkey (Finn et al., 2001), strongly implying a serotonergic modulatory role for leptin in anxiety.

Other lines of evidence indirectly link 5-HT to leptin. The 5-HT transporter, which is targeted by Serotonin-Specific Reuptake Inhibitors, is linked to obesity (Üçeyler et al., 2010). There is also evidence that polymorphisms of the 5-HT receptor are linked to anorexia and bulimia (Collier et al., 1997; Nacmias et al., 1999). This suggests that disorders of eating behaviour which are modulated by leptin, amongst other things, are linked to the 5-HT system.

With increasing evidence of a connection between leptin and the 5-HT system, as well as studies supporting its anxiolytic potential, and the established role for 5-HT agonists as anxiolytics, the inconsistent performance of leptin in behavioural assays of anxiety is perplexing. The open field and elevated plus maze tests used to assess leptin also produce inconsistent results when testing well-established clinical anxiolytics, not just with regard to failure to detect anxiolytic potential in known anxiolytics, but with some non-anxiolytic compounds being incorrectly detected as anxiolytic (for review, see (Crawley, 1985; Lister, 1990).

In contrast to these spatial exploratory paradigms, which can produce mixed results, the Fixed Interval 60 Seconds (FI60) is an operant behavioural task that involves non-spatial behavioural inhibition. It assesses response suppression in a similar manner to the Vogel conditioned suppression of licking, Geller-Seifter, and Conditioned Emotional Response tests, but has the advantage of suppressing responding with the aversive state of frustration and not using shock and so not involving a confound with any analgesic action a drug may have. Fear and frustration have similar eliciting properties and their suppression of responding is similarly sensitive to anxiolytic drugs (Gray, 1977). Control animals in the FI60 paradigm rapidly learn to inhibit the bulk of their responding until after the 60-second non-reward period has elapsed. However, following administration of an anxiolytic drug animals are typically less able to inhibit their behaviour, making a larger number of inappropriately early responses during the non-reward period. This is true for all anxiolytics so far tested, regardless of precise pharmacological mode of action (Munn and McNaughton, 2008; Panickar and McNaughton, 1991; Zhu and McNaughton, 1995). One further advantage of the FI60 paradigm is that it provides an indirect assay of pharmacological mode of action. GABA-ergic anxiolytics such as the barbiturates and benzodiazepines show a linear dose–response relationship in the FI60; larger doses typically produce a larger release of responding. In contrast, the serotonergic anxiolytics so far tested tend to show an inverted-U dose–response curve; low doses produce a release of responding, while higher doses suppress responding (Munn and McNaughton, 2008; Panickar and McNaughton, 1991). It should be noted that while the form of the dose–response curve is different for the different classes of drugs, the low dose effects all involve a similar release of inhibition in the initial period of responding and the differences between the classes disappear with long-term administration (Zhu and McNaughton, 1995).

The aim of the present study is therefore twofold. First, given the inconsistent results produced by standard tests of anxiety, we first aim to examine the anxiolytic potential of leptin in the more reliable FI60. Secondly, should leptin behave as an anxiolytic in the FI60, we predict that it will show a dose response curve consistent with 5HT (i.e. response depression at higher doses) rather than GABA agency.

2. Methods

2.1. Subjects

The subjects were 24 naïve male Sprague–Dawley rats, obtained from the University of Otago Department of Laboratory Animal Sciences. They weighed between 243 and 323 g immediately prior to the experiments. The home room was maintained on a 12 h light, 12 h dark cycle; (lights on at 0600 and off at 1800). The temperature of the home room was maintained at 20–22 °C. Animals in all cages had access to water and Reliance stock food pellets ad libitum.

The animals were initially housed in 49 cm × 31 cm × 26.5 cm cages, in groups of four. Twelve days before the pre-training sessions began the animals were taken off ad libitum access to food and placed on a restricted diet of Reliance Stock food pellets. Animals were weighed daily, and the amount of food they received each day was varied in order to maintain their weight at 80% of their free feeding weight. Animals had ad libitum access to water throughout the experiment.

2.2. Apparatus

Six standard operant chambers (Campden Instruments, U.K.) were used throughout the training sessions as well as all the experimental trials. The dimensions of the operant chambers were 57.5 cm × 34.5 cm × 39 cm. The interior of the operant chambers contained a smaller interior chamber which was 24 cm × 24 cm × 26 cm. A food delivery system distributed individual 45 mg Dustless Precision Pellets (Campden Instruments Ltd., UK) to a food hopper in the interior chamber through a plastic tube. The front-facing wall of the operant chamber was hinged from the bottom in order to allow access to the interior. One of the long exterior walls of the chamber had a circular tinted window 16.6 cm in diameter, which allowed a view of the interior of the chamber.

The interior chamber of the operant chamber had three metal walls, a metal ceiling, and a horizontal grid floor. The front-facing wall of the interior chamber was a transparent Perspex wall that could be unlatched from the top of the box and pulled down to allow access to the chamber. One of the walls had a recessed 5 cm by 6.5 cm food hopper with a Perspex door hinged from the top, the food hopper was set in the middle and at the bottom of the wall. Two retractable metal levers were set into the wall, one on either side of, and equidistant from, the hopper. Only the left hand lever was extended into the chamber in the current experiment. Directly above each lever there were two 2.8 W stimulus lights set into the wall. The other two metal walls of the housing chamber were bare. In the centre of the metal ceiling of the housing chamber there was a 2.8 W house light, which was on throughout the training and experimental trials. There was also an electric fan, which provided ventilation for the animals, and also produced a constant level of background noise.

Three IBM compatible computers, which were running Visual Basic 6 software with LABJACK control components, each controlled two operant chambers. Custom Visual Basic 6 programs were used to deliver the three training schedules. This meant that the computers controlled the timing and delivery of the reinforcements, as well as recording the number of lever presses the animal made, and the number of nose pokes into the food hopper. The computers also recorded the time each lever press was made during the FI60 task. These responses were divided into one of twelve five-second bins, according to the interval since the last reward that they were made. Responses made between 0 and 5 s since the last reward were allocated to bin one; responses made between 5 and 10 s since the last reward were allocated to bin two and so on.